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The formation of bacterial endospores in the living animal body has not been reported. [In 1959-1960, M. Taylor, Fort Detrick, observed (*unpublished data*), through several weeks, anthrax spores produced in the cecum of a germ-free Lohund rat after challenge.] General opinion is that it does not occur. We have found, however, that in vivo-formed spores of several species of bacilli may be demonstrated in the chicken egg embryo.

Bacillus anthracis, strains Vollum and 30R, and *B. subtilis* var. *niger* were inoculated into the yolk sac of antibiotic-free eggs containing 9-day-old embryos. The inoculum consisted solely of spores at a dose level of less than 100 spores per egg (1 to 2 spores/ml). After 22 hr of incubation at 37 C, the eggs were candled and those in which movement was observed were harvested. At least six eggs were blended in a Waring-type blender for whole-egg slurry, and an equal number was harvested selectively for embryo, yolk, and allantoic fluid. These samples were pooled, blended, and assayed immediately for total and spore count. The cells were surface-plated on Tryptose Agar (Difco). Vegetative cells were destroyed by heating at 65 C for 30 min. The data given are an average of five replicates.

The average increase in numbers of spores per ml in whole eggs was about 4,500 to 9,000 times for *B. anthracis* (Vollum), 200 to 400 times for *B. anthracis* (30R), and 4,000 to 8,000 times for *B. subtilis* var. *niger* (Table 1). Portions of the egg, particularly the allantoic fluid, contained greater numbers of in vivo-formed spores than did other selectively harvested portions. The spores were easily visualized in stained smears of the various selectively harvested portions of the eggs. Figure 1 shows a smear prepared from the allantoic fluid. Spores are seen as clear, swollen areas in the sporangium. Variation in total cell and spore counts was large between replicates, and the number of cells in the selectively harvested portion might vary significantly from the number in the blended whole egg.

The morphology of colonies grown from spores formed in vivo indicated that no genetic change had occurred as a result of growth and sporulation in the egg.

Clostridium botulinum, types A and B, and *Bacillus popilliae* var. *dutky* also were cultivated in chick embryos. Although both formed spores, neither could be said to have produced them in vivo. *C. botulinum* required nonviable embryos for growth; *B. popilliae*, to reach its maximal

TABLE 1. *Total viable count and viable spore count of some Bacillus species grown in vivo in chick embryos*

Organism	Egg menstra	Total viable count (10^8/ml)	Viable spore count (10^8/ml)	Factorial increase in spores over inoculum
B. anthracis Vollum	Whole egg slurry	59.7	0.09	4,500–9,000
	Allantoic fluid	173.9	3.63	181,500–363,000
	Embryo slurry	110.6	0.07	3,500–7,000
	Yolk	18.7	0.05	2,500–5,000
B. anthracis 30R	Whole egg slurry	266.7	0.004	200–400
	Allantoic fluid	36.7	0.003	150–300
	Embryo slurry	78.6	0.003	150–300
	Yolk	88.1	0.01	500–1,000
B. subtilis var. *niger*	Whole egg slurry	0.6	0.08	4,000–8,000
	Allantoic fluid	552.4	0.20	14,500–29,000
	Embryo slurry	194.4	0.13	6,500–13,000
	Yolk	893.5	0.09	4,500–9,000

FIG. 1. *In vivo-formed spores of Bacillus anthracis in allantoic fluid from live chick embryos.*

population level, required an incubation time beyond the viability limit of the embryos. *C. botulinum* proceeded to complete sporulation in 72 hr, but the greatest percentage sporulation observed with *B. popilliae* was about 0.1% in a population of 189 million cells/ml after 6 days of incubation at 30 C.

Lack of sporogenesis of *B. anthracis* in the animal body usually is attributed to oxygen imbalance; however, a mineral imbalance seems equally likely. The high lysozyme content of the egg, which might affect membrane permeability, and the fact that anthrax is not established in the adult chicken unless body temperature is lowered, are, at present, unrelated observations, but ones suggesting that the use of the chick embryo as a new approach to studies on growth, sporogenesis, and pathogenesis of *B. anthracis* may lead to new information on the development and evaluation of these processes.